	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
1	BRS	L1	1254	fluorogenic adj (peptide or substrate)	USPAT; US-PGPUB; EPO; DERWENT	2002/07/27 11:12			0
2	BRS	L2	48250	protease or proteinase	USPAT; US-PGPUB; EPO; DERWENT	2002/07/27 10:57			0
3	BRS	L3	267	1 same 2	USPAT; US-PGPUB; EPO; DERWENT	2002/07/27 10:57			0
4	BRS	L4	0	3 same rh110	USPAT; US-PGPUB; EPO; DERWENT	2002/07/27 10:57			0
5	BRS	L5	22	3 same rhodamine	USPAT; US-PGPUB; EPO; DERWENT	2002/07/27 11:07			0
6	BRS	L7	10	fluorogenic adj composition	USPAT; US-PGPUB; EPO; DERWENT	2002/07/27 11:15			0
7	BRS	L8	7	2 same 7	USPAT; US-PGPUB; EPO; DERWENT	2002/07/27 11:16			0

=> d his

(FILE 'HOME' ENTERED AT 11:21:13 ON 27 JUL 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
11:21:42 ON 27 JUL 2002
L1 5943 S FLUOROGENIC (W) (SUBSTRATE OR PEPTIDE OR COMPOSITION)
L2 456506 S PROTEASE OR PROTEINASE
L3 1780 S L1 (P) L2
L4 40842 S RHODAMINE
L5 31 S L3 (P) L4
L6 10 DUPLICATE REMOVE L5 (21 DUPLICATES REMOVED)
L7 2 S FLUOROGENIC (W) COMPOSITION
L8 2 DUPLICATE REMOVE L7 (0 DUPLICATES REMOVED)
L9 212 S KOMORIYA A/AU
L10 13 S L9 AND FLUOROGENIC
L11 7 DUPLICATE REMOVE L10 (6 DUPLICATES REMOVED)

```
FILE 'HOME' ENTERED AT 11:21:13 ON 27 JUL 2002

=> file medline caplus biosis embase scisearch agricola
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'MEDLINE' ENTERED AT 11:21:42 ON 27 JUL 2002

FILE 'CAPLUS' ENTERED AT 11:21:42 ON 27 JUL 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 11:21:42 ON 27 JUL 2002
COPYRIGHT (C) 2002 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'EMBASE' ENTERED AT 11:21:42 ON 27 JUL 2002
COPYRIGHT (C) 2002 Elsevier Science B.V.  All rights reserved.

FILE 'SCISEARCH' ENTERED AT 11:21:42 ON 27 JUL 2002
COPYRIGHT (C) 2002 Institute for Scientific Information (ISI) (R)

FILE 'AGRICOLA' ENTERED AT 11:21:42 ON 27 JUL 2002

=> s fluorogenic (w) (substrate or peptide or composition)
L1        5943 FLUOROGENIC (W) (SUBSTRATE OR PEPTIDE OR COMPOSITION)

=> s protease or proteinase
L2      456506 PROTEASE OR PROTEINASE

=> s l1 (p) l2
L3        1780 L1 (P) L2

=> s rhodamine
L4       40842 RHODAMINE

=> s l3 (p) l4
L5          31 L3 (P) L4

=> duplicate remove l5
DUPLICATE PREFERENCE IS 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH'
KEEP DUPLICATES FROM MORE THAN ONE FILE?  Y/(N):n
PROCESSING COMPLETED FOR L5
L6           10 DUPLICATE REMOVE L5 (21 DUPLICATES REMOVED)

=> d l6 1-10 ibib abs

L6   ANSWER 1 OF 10  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:         2001:185948  CAPLUS
DOCUMENT NUMBER:          134:248826
TITLE:                    Fluorogenic peptides for the detection of protease
                          activity in biological samples and methods of their
                          use
INVENTOR(S):              Komoriya, Akira; Packard, Beverly S.
PATENT ASSIGNEE(S):       Oncoimmunin, Inc., USA
SOURCE:                   PCT Int. Appl., 86 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   1
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
     ---------------   ----  --------       ---------------  --------
     WO 2001018238      A1   20010315       WO 2000-US24882  20000911
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
             CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
             HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
             LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
```

SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1214445 A1 20020619 EP 2000-961782 20000911
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO, MK, CY, AL

PRIORITY APPLN. INFO.: US 1999-394019 A 19990910
WO 2000-US24882 W 20000911

OTHER SOURCE(S): MARPAT 134:248826

AB The present invention provides for novel reagents whose fluorescence increases in the presence of particular proteases. The reagents comprise a characteristically folded peptide backbone conjugated to two fluorophores such that the fluorophores are located opposite sides of a cleavage site. When the folded peptide is cleaved, as by digestion with a protease, the fluorophores provide a high intensity fluorescent signal at a visible wavelength. Because of their high fluorescence signal in the visible wavelengths, these protease indicators are particularly well suited for detection of protease activity in biol. samples, in particular in frozen tissue sections. Thus, this invention also provides for methods of detecting protease activity in situ in frozen sections.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 2 OF 10 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2001666338 MEDLINE
DOCUMENT NUMBER: 21540684 PubMed ID: 11683632
TITLE: Human adenovirus proteinase: DNA binding and stimulation of proteinase activity by DNA.
AUTHOR: McGrath W J; Baniecki M L; Li C; McWhirter S M; Brown M T; Toledo D L; Mangel W F
CORPORATE SOURCE: Biology Department, Brookhaven National Laboratory, Upton, New York 11973, USA.
CONTRACT NUMBER: AI41599 (NIAID)
SOURCE: BIOCHEMISTRY, (2001 Nov 6) 40 (44) 13237-45.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011120
Last Updated on STN: 20020123
Entered Medline: 20011207

AB The interaction of the human adenovirus ***proteinase*** (AVP) with various DNAs was characterized. AVP requires two cofactors for maximal activity, the 11-amino acid residue peptide from the C-terminus of adenovirus precursor protein pVI (pVIc) and the viral DNA. DNA binding was monitored by changes in enzyme activity or by fluorescence anisotropy. The equilibrium dissociation constants for the binding of AVP and AVP-pVIc complexes to 12-mer double-stranded (ds) DNA were 63 and 2.9 nM, respectively. DNA binding was not sequence specific; the stoichiometry of binding was proportional to the length of the DNA. Three molecules of the AVP-pVIc complex bound to 18-mer dsDNA and six molecules to 36-mer dsDNA. When AVP-pVIc complexes bound to 12-mer dsDNA, two sodium ions were displaced from the DNA. A Delta of -4.6 kcal for the nonelectrostatic free energy of binding indicated that a substantial component of the binding free energy results from nonspecific interactions between the AVP-pVIc complex and DNA. The cofactors altered the interaction of the enzyme with the ***fluorogenic*** ***substrate*** (Leu-Arg-Gly-Gly-NH)2-***rhodamine*** . In the absence of any cofactor, the Km was 94.8 microM and the kcat was 0.002 s(-1). In the presence of adenovirus DNA, the Km decreased 10-fold and the kcat increased 11-fold. In the presence of pVIc, the Km decreased 10-fold and the kcat increased 118-fold. With both cofactors present, the kcat/Km ratio increased 34000-fold, compared to that with AVP alone. Binding to DNA was coincident with stimulation of ***proteinase*** activity by DNA. Although other ***proteinases*** have been shown to bind to DNA, stimulation of ***proteinase*** activity by DNA is unprecedented. A model is presented suggesting that AVP moves along the viral DNA looking for precursor protein cleavage sites much like RNA polymerase moves along DNA looking for a promoter.

L6 ANSWER 3 OF 10 MEDLINE DUPLICAT
ACCESSION NUMBER: 2001136605 MEDLINE
DOCUMENT NUMBER: 20537044 PubMed ID: 11084874
TITLE: Sensitive method to identify and characterize proteinases in situ after SDS-PAGE.
AUTHOR: Williams J; McGrath W J; Mangel W F
CONTRACT NUMBER: AI41599 (NIAID)
SOURCE: BIOTECHNIQUES, (2000 Nov) 29 (5) 1108-13.
Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20010404
Entered Medline: 20010301

AB Cells and body fluids contain numerous, different ***proteinases*** ; to identify and characterize them are both important and difficult tasks. Especially difficult to identify and characterize are highly specific ***proteinases*** . Here, we present an extremely sensitive and quantitative method to characterize ***proteinases*** fractionated by SDS-PAGE that cleave specific ***rhodamine*** -based ***fluorogenic*** ***substrates*** . To test the sensitivity of the technique, we used trypsin as our model system. Filter paper impregnated with ***rhodamine*** -based ***fluorogenic*** ***substrates*** was placed on a gel, and bands of fluorescence originating from specific ***proteinases*** were visualized in real time. The method is very sensitive; picogram amounts of trypsin can be detected. The method should be very general, in that even ***proteinases*** whose substrates require amino acids C-terminal to the cleavage site may be identified and characterized. The results allow one to obtain not only information on the substrate specificity of a specific enzyme but also information about its molecular weight.

L6 ANSWER 4 OF 10 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001115034 MEDLINE
DOCUMENT NUMBER: 21014629 PubMed ID: 11131845
TITLE: Flow cytometric analysis of enzymes in live spermatozoa before and after cryostorage.
AUTHOR: Schaller J; Glander H J
CORPORATE SOURCE: Department of Dermatology, St. Barbara Hospital, Duisburg, Germany.
SOURCE: ANDROLOGIA, (2000 Nov) 32 (6) 357-64.
Journal code: 0423506. ISSN: 0303-4569.
PUB. COUNTRY: Germany: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010215

AB Synthetic ***fluorogenic*** ***substrates*** , like the CellProbe reagents, can determine enzymes in vital human spermatozoa. These substrates will enter the cells without previous cell permeabilization and exhibit fluorescence after cleavage depending on enzyme activity. They consist of different peptide sequences, specific for the enzymes, and a fluorescein- or ***rhodamine*** 110-dye moiety. The number of positive cells and the intensity of the fluorescence can be determined by flow cytometric analysis. We investigated several enzymes (peptidases, ***proteinases*** , esterases, elastases and collagenases) in intact spermatozoa before and after cryoprotection. Semen samples with normal spermiogram parameters were cryoprotected using the freezing medium TEST yolk buffer (TYB). Fresh spermatozoa showed a marked fluorescence after incubation with the synthetic substrates for the aminopeptidase M, butyryl esterase, fluorescein diacetate (FDA)-and FDA/sodium fluoride (NAF)-esterase, ala-ala-pro-val (AAPV)-elastase, gly pro-leu-gly pro-(GPLGP)-collagenase, gly gly leu-(GGL)-subtilisin as well as lys-ala-(LA)-dipeptidyl peptidase (DPP) II. After cryopreservation the spermatozoal fluorescence increased applying substrates for butyryl

esterase (P<0.05), prolyl-aminopeptidase (P<0.001) and val-lys-(VK)-cathepsin (P<0.01) most probably due to elevated enzyme activities. The activities of FDA-esterase (P<0.05) and FDA/NAF-esterase (P<0.05), AAPV-elastase (P<0.01), GPLGP-collagenase (P<0.05) and GGL-subtilisin (P<0.001) decreased after cryopreservation. The substrates for arg-gly glut-ser-(RGES)-elastase, gly phenyl-gly ala-(GFGA)-collagenase and threo-pro-(TP)-cathepsin were not cleaved before as well as after cryostorage. The substrates for subtilisin an

L6 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:930260 CAPLUS
DOCUMENT NUMBER: 123:333536
TITLE: Functional characterization of the adenovirus proteinase using fluorogenic substrates
AUTHOR(S): Diouri, Mounir; Geoghegan, Kieran F.; Weber, Joseph M.
CORPORATE SOURCE: Dep. Microbiol., Univ. Sherbrooke, PQ, J1H 5N4, Can.
SOURCE: Protein Pept. Lett. (1995), 2(2), 363-70
CODEN: PPELEN; ISSN: 0929-8665
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Continuous fluorometric assays with 2 different substrates were used to extend functional characterization of the cysteine proteinase from adenovirus. Among the effects studied were the NaCl concn., the addn. of DNA, and the putative activating peptide pVIct (GVQSLKRRRCF). In addn., it was shown that the specific activities of both wild-type enzyme and a mutant proteinase from a form of the virus in which maturation was temp.-sensitive were elevated by a similar factor at the nonpermissive temp. of 39.degree.. This observation supported an earlier demonstration that the mutant proteinase from the temp.-sensitive (ts) form of the virus is not temp.-sensitive in vitro. It was consistent with the concept that temp.-sensitivity arises from a fault in protein trafficking at nonpermissive temps.

L6 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1993:208761 CAPLUS
DOCUMENT NUMBER: 118:208761
TITLE: Biochemical parameters of cell function
AUTHOR(S): Rothe, G.; Valet, G.
CORPORATE SOURCE: Klin., Univ. Regensburg, Regensburg, 8400, Germany
SOURCE: Flow Cytom. Cell Sorting (1992), 100-20. Editor(s): Radbruch, Andreas. Springer: Berlin, Germany.
CODEN: 58UIA7
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Functional parameters are analyzed by flow cytometry following staining of vital cells with specific fluorescent or fluorogenic indicators. Intracellular ion concns. such as the intracellular pH value and the cytosolic free calcium concn. are measured by changes in the fluorescence emission spectrum or intensity of ion-sensitive probes such as 2,3-dicyanohydroquinone, carboxy-seminaphthorhodafluor-1, indo-1, or fluo-3. The membrane potential of plasma or mitochondrial membrane can be measured by the accumulation of lipophilic fluorescent indicator dyes with a delocalized charge. Cellular oxidants such as superoxide anion or hydrogen peroxide or antioxidants such as glutathione can be measured by the formation of specific fluorescent products with ***fluorogenic*** ***substrates*** . Specific enzymic activities such as lysosomal ***protease*** activities can be analyzed by the fluorescence generated by the intracellular cleavage of specifically N,N'-bis-peptide substituted ***rhodamine*** 110 (R110) derivs.

L6 ANSWER 7 OF 10 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1987:133823 BIOSIS
DOCUMENT NUMBER: BR32:62458
TITLE: NOVEL ***RHODAMINE*** DERIVATIVES AS ***FLUOROGENIC*** ***SUBSTRATES*** FOR ***PROTEINASES*** .
AUTHOR(S): MANGEL W F; LEYTUS S; MELHADO L L
CORPORATE SOURCE: URBANA, ILL., USA.
ASSIGNEE: UNIVERSITY OF ILLINOIS
PATENT INFORMATION: US 4640893 03 Feb 1987
SOURCE: Off. Gaz. U. S. Pat. Trademark Off., Pat., (1987) 1075 (1), 337.

CODEN: OGUPEZ. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
FILE SEGMENT: BR; OLD
LANGUAGE: English

L6 ANSWER 8 OF 10 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 84257685 MEDLINE
DOCUMENT NUMBER: 84257685 PubMed ID: 6204689
TITLE: Theory and experimental method for determining individual kinetic constants of fast-acting, irreversible proteinase inhibitors.
AUTHOR: Leytus S P; Toledo D L; Mangel W F
CONTRACT NUMBER: CA 25633 (NCI)
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1984 Jul 17) 788 (1) 74-86.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198409
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 20000303
Entered Medline: 19840905
AB A theory and experimental method are presented to characterize the kinetics of fast-acting, irreversible ***proteinase*** inhibitors. The theory is based upon formal analysis of the case of an irreversible inhibitor competing with a substrate for the active-site of a ***proteinase*** . From this theory, an experimental method is described by which the individual microscopic kinetic constants for the interaction of the inhibitor with the ***proteinase*** can be determined. These are, for a two-step inhibition reaction sequence, the equilibrium dissociation constant and the first-order rate constant for inhibition, and, for a one-step inhibition reaction sequence, the second-order rate constant for inhibition. The theory and experimental method were validated by an analysis of the inhibition of trypsin by the two-step synthetic inhibitor p-nitrophenyl p-guanidinobenzoate and the one-step protein inhibitor bovine pancreatic trypsin inhibitor. The substrate used in these experiments is a new, ***fluorogenic*** ***substrate*** for trypsin-like serine ***proteinases*** (Cbz-Ile-Pro-Arg-NH)2- ***Rhodamine*** , the synthesis and properties of which are described.

L6 ANSWER 9 OF 10 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 83204009 MEDLINE
DOCUMENT NUMBER: 83204009 PubMed ID: 6342611
TITLE: ***Rhodamine*** -based compounds as ***fluorogenic*** ***substrates*** for serine ***proteinases*** .
AUTHOR: Leytus S P; Melhado L L; Mangel W F
CONTRACT NUMBER: CA 25633 (NCI)
SOURCE: BIOCHEMICAL JOURNAL, (1983 Feb 1) 209 (2) 299-307.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198306
ENTRY DATE: Entered STN: 19900318
Last Updated on STN: 20000303
Entered Medline: 19830610
AB A new ***fluorogenic*** ***substrate*** for serine ***proteinases*** , bis(N-benzyloxycarbonyl-L-argininamido) ***Rhodamine*** [(Cbz-Arg-NH)2- ***Rhodamine***], was synthesized, purified and chemically and enzymically characterized. This compound, which employs ***Rhodamine*** as a fluorophoric leaving group, is the first in a series of substrates designed to measure the amidase activity of ***proteinases*** . Cleavage of one of the amide bonds of (Cbz-Arg-NH)2- ***Rhodamine*** by a trypsin-like serine ***proteinase*** converts the non-fluorescent bisamide substrate into a highly fluorescent monoamide product. Significant differences in the electronic absorption and fluorescence emission spectra and quantum yields of bis-, mono- and un-substituted ***Rhodamine*** are reported. Macroscopic kinetic constants for the interaction of (Cbz-Arg-NH)2- ***Rhodamine*** with bovine trypsin, human and dog plasmin and human

thrombin were determined. Compared with the corresponding 7-amino-4-methylcoumarin-based analogue, (Cbz-Arg-NH)2- ***[redacted]odamine*** exhibits an increase in sensitivity with these enzymes of 50--300-fold. The physical basis for this increase in sensitivity is discussed.

L6 ANSWER 10 OF 10 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 84079700 MEDLINE
DOCUMENT NUMBER: 84079700 PubMed ID: 6228222
TITLE: New class of sensitive and selective ***fluorogenic*** ***substrates*** for serine ***proteinases*** . Amino acid and dipeptide derivatives of ***rhodamine*** .
AUTHOR: Leytus S P; Patterson W L; Mangel W F
CONTRACT NUMBER: CA 25633 (NCI)
SOURCE: BIOCHEMICAL JOURNAL, (1983 Nov 1) 215 (2) 253-60.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198401
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 20000303
Entered Medline: 19840107

AB A series of dipeptide derivatives of Rhodamine, each containing an arginine residue in the P1 position and one of ten representative benzyloxycarbonyl (Cbz)-blocked amino acids in the P2 position, has been synthesized, purified and characterized as substrates for serine proteinases. These substrates are easily prepared with high yields. Cleavage of a single amide bond converts the non-fluorescent bisamide substrate into a highly fluorescent monoamide product. Macroscopic kinetic constants for the interaction of these substrates with bovine trypsin, human and dog plasmin, and human thrombin are reported. Certain of these substrates exhibit extremely large specificity constants. For example, the kcat./Km for bovine trypsin with bis-(N-benzyloxycarbonylglycyl-argininamido)-Rhodamine [(Cbz-Gly-Arg-NH)2-Rhodamine] is 1 670 000 M-1 X S-1. Certain of these substrates are also highly selective. For example, the most specific substrate for human plasmin, (Cbz-Phe-Arg-NH2)-Rhodamine, is not hydrolysed by human thrombin, and the most specific substrate for human thrombin, (Cbz-Pro-Arg-NH)2-Rhodamine, is one of the least specific substrates for human plasmin. Comparison of the kinetic constants for hydrolysis of the dipeptide substrates with that of the single amino acid derivative, (Cbz-Arg-NH)2-Rhodamine, indicates that selection of the proper amino acid residue in the P2 position can effect large increases in substrate specificity. This occurs primarily as a result of an increase in kcat. as opposed to a decrease in Km and, in certain cases, is accompanied by a large increase in selectivity. Because of their high degree of sensitivity and selectivity, these Rhodamine-based dipeptide compounds should be extremely useful substrates for studying serine proteinases.

```
=> d his

     (FILE 'HOME' ENTERED AT 11:21:13 ON 27 JUL 2002)

     FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
     11:21:42 ON 27 JUL 2002
L1          5943 S FLUOROGENIC (W) (SUBSTRATE OR PEPTIDE OR COMPOSITION)
L2        456506 S PROTEASE OR PROTEINASE
L3          1780 S L1 (P) L2
L4         40842 S RHODAMINE
L5            31 S L3 (P) L4
L6            10 DUPLICATE REMOVE L5 (21 DUPLICATES REMOVED)

=> s FLUOROGENIC (W) COMPOSITION
L7             2 FLUOROGENIC (W) COMPOSITION

=> duplicate remove l7
PROCESSING COMPLETED FOR L7
L8             2 DUPLICATE REMOVE L7 (0 DUPLICATES REMOVED)

=> d l8 1-2 ibib abs
```

L8 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS I
ACCESSION NUMBER: 2002:101996 BIOSIS
DOCUMENT NUMBER: PREV200200101996
TITLE: Compositions for the detection of protease in biological samples and methods of use therefo.
AUTHOR(S): Komoriya, A.; Parkard, B. S.
CORPORATE SOURCE: Rockville, Md. USA
ASSIGNEE: ONCOIMMUNIN, INC.
PATENT INFORMATION: US 5714342 Feb. 3, 1998
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 3, 1998) Vol. 1207, No. 1, pp. 412. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

L8 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:62138 BIOSIS
DOCUMENT NUMBER: PREV200200062138
TITLE: Compositions for the detection of proteases in biological samples and methods of use thereof.
AUTHOR(S): Komoriya, A.; Packard, B. S.
CORPORATE SOURCE: Rockville, Md. USA
ASSIGNEE: ONCOIMMUNIN, INC.
PATENT INFORMATION: US 5605809 Feb. 25, 1997
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 25, 1997) Vol. 1195, No. 4, pp. 2522. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

=> s komoriya A/au
L9 212 KOMORIYA A/AU

=> d his

(FILE 'HOME' ENTERED AT 11:21:13 ON 27 JUL 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 11:21:42 ON 27 JUL 2002
L1 5943 S FLUOROGENIC (W) (SUBSTRATE OR PEPTIDE OR COMPOSITION)
L2 456506 S PROTEASE OR PROTEINASE
L3 1780 S L1 (P) L2
L4 40842 S RHODAMINE
L5 31 S L3 (P) L4
L6 10 DUPLICATE REMOVE L5 (21 DUPLICATES REMOVED)
L7 2 S FLUOROGENIC (W) COMPOSITION
L8 2 DUPLICATE REMOVE L7 (0 DUPLICATES REMOVED)
L9 212 S KOMORIYA A/AU

=> s l9 and fluorogenic
L10 13 L9 AND FLUOROGENIC

=> duplicate remove l10
DUPLICATE PREFERENCE IS 'MEDLINE, BIOSIS, EMBASE, SCISEARCH'
KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L10
L11 7 DUPLICATE REMOVE L10 (6 DUPLICATES REMOVED)

=> d l11 1-7 ibib abs

L11 ANSWER 1 OF 7 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 1
ACCESSION NUMBER: 2002068400 EMBASE
TITLE: Visualization and quantification of T cell-mediated cytotoxicity using cell-permeable ***fluorogenic*** caspase substrates.
AUTHOR: Liu L.; Chahroudi A.; Silvestri G.; Wernett M.E.; Kaiser W.J.; Safrit J.T.; ***Komoriya A.*** ; Altman J.D.; Packard B.Z.; Feinberg M.B.
CORPORATE SOURCE: M.B. Feinberg, Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA, United States.

mbf@sph.emory.edu
SOURCE: Nature Medicine, (2002) 8/2 (185-189).
Refs: 20
ISSN: 1078-8956 CODEN: NAMEFI
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We have developed a non-radioactive flow-cytometry assay to monitor and quantify the target-cell killing activities mediated by cytotoxic T lymphocytes (CTLs). This flow-cytometry CTL (FCC) assay is predicated on measurement of CTL-induced caspase activation in target cells through detection of the specific cleavage of ***fluorogenic*** caspase substrates. Here we show that this assay reliably detects antigen-specific CTL killing of target cells, and demonstrate that it provides a more sensitive, more informative and safer alternative to the standard (51)Cr-release assay most often used to quantify CTL responses. The FCC assay can be used to study CTL-mediated killing of primary host target cells of different cell lineages, and enables the study of antigen-specific cellular immune responses in real time at the single-cell level. As such, the FCC assay can provide a valuable tool for studies of infectious disease pathogenesis and development of new vaccines and immunotherapies.

L11 ANSWER 2 OF 7 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 2002050858 EMBASE
TITLE: Detection of localized caspase activity in early apoptotic cells by laser scanning cytometry.
AUTHOR: Telford W.G.; ***Komoriya A.*** ; Packard B.Z.
CORPORATE SOURCE: Dr. W.G. Telford, National Cancer Institute, Medicine Branch Building 10, 9000 Rockville Pike, Bethesda, MD 20892, United States. telfordw@box-t.nih.gov
SOURCE: Communications in Clinical Cytometry, (1 Feb 2002) 47/2 (81-88).
Refs: 27
ISSN: 0196-4763 CODEN: CCCYEM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Background: Caspase activation is a critical early step in the onset of apoptosis. Cell-permeable ***fluorogenic*** caspase substrates have proven valuable in detecting caspase activation by flow cytometry. Nevertheless, detection of early low-level caspase activation has been difficult using conventional area or peak fluorescence analysis by flow cytometry, despite the apparent presence of these cells as observed by microscopy. We describe a method utilizing maximum fluorescence pixel analysis by laser scanning cytometry (LSC) to detect early apoptotic cells. Methods: The PhiPhiLux-G(1)D(2) caspase 3/7 substrate was used in combination with DNA dye exclusion and annexin V binding to identify several stages of apoptosis in EL4 murine thymoma cells by both traditional flow and LSC. LSC analysis of maximum pixel brightness in individual cells demonstrated an intermediate caspase-low subpopulation not detectable by flow or LSC integral analysis. LSC analysis of caspase activity was then carried out using the larger UMR-106 rat osteosarcoma cell line to determine if this apparent early caspase activity could be correlated with localized, punctate caspase activity observed by microscopy. Results: The caspase-low subpopulation found in apoptotic EL4 cells was also observable in UMR-106 cells. Relocation to cells with low fluorescence due to caspase activity and subsequent examination by microscopy demonstrated that these latter cells indeed show punctate, highly localized caspase activation foci that might represent an early stage in caspase activation. Conclusions: Cells with low-level, localized caspase expression can be detected using maximum pixel analysis by LSC. This methodology allows an early step of apoptotic activation to be resolved for further analysis. .COPYRGT. 2002 Wiley-Liss, Inc.

L11 ANSWER 3 OF 7 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 2001567365 MEDLINE

DOCUMENT NUMBER: 21528940 PubMed ID: 11673515
TITLE: Caspase 8 activity in membrane blebs after anti-Fas ligation.
AUTHOR: Packard B Z; ***Komoriya A*** ; Brotz T M; Henkart P A
CORPORATE SOURCE: OncoImmunin, Inc., Gaithersburg, MD 20877, USA.
SOURCE: JOURNAL OF IMMUNOLOGY, (2001 Nov 1) 167 (9) 5061-6.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200112
ENTRY DATE: Entered STN: 20011024
Last Updated on STN: 20020122
Entered Medline: 20011205

AB Previous studies of thymocyte apoptosis using a series of cell-permeable ***fluorogenic*** peptide substrates showed that Fas cross-linking triggered a caspase cascade in which cleavage of the IETDase (caspase 8-selective) substrate was the earliest caspase activity measured by flow cytometry. This result was expected in light of the abundant evidence for caspase 8 activation as an initiating event in the Fas death pathway. However, when apoptosis was induced by anti-Fas in CTL and the caspase cascade examined by this approach, IETDase activation followed increases in LEHDase, YVHDase, and VEIDase activities (selective for caspases 9, 1, and 6, respectively). When examined by confocal microscopy, anti-Fas-treated CTL showed the early appearance of IETDase-containing plasma membrane vesicles and their release from the CTL surface, followed by activation of other caspase activities in the cell interior. Since these vesicles were not included in the flow cytometry analysis, the early IETDase activity had been underestimated. In contrast to anti-Fas, induction of apoptosis in these CTL by IL-2 withdrawal resulted in early IETDase activity in the cytoplasm, with no plasma membrane vesiculation. Thus, anti-Fas-induced initiation of caspase activity at the plasma membrane may in some cells result in local proteolysis of submembrane proteins, leading to generation of membrane vesicles that are highly enriched in active caspase 8.

L11 ANSWER 4 OF 7 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2000298890 MEDLINE
DOCUMENT NUMBER: 20298890 PubMed ID: 10839799
TITLE: Assessment of caspase activities in intact apoptotic thymocytes using cell-permeable ***fluorogenic*** caspase substrates.
AUTHOR: ***Komoriya A*** ; Packard B Z; Brown M J; Wu M L; Henkart P A
CORPORATE SOURCE: OncoImmunin, Incorporated, Gaithersburg, MD 20877, USA.
SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, (2000 Jun 5) 191 (11) 1819-28.
Journal code: 2985109R. ISSN: 0022-1007.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000811
Last Updated on STN: 20000811
Entered Medline: 20000803

AB To detect caspase activities in intact apoptotic cells at the single cell level, cell-permeable ***fluorogenic*** caspase substrates were synthesized incorporating the optimal peptide recognition motifs for caspases 1, 3/7, 6, 8, and 9. Caspase activities were then assessed at various times after in vitro treatment of mouse thymocytes with dexamethasone or anti-Fas antibody. Dexamethasone induced the following order of appearance of caspase activities as judged by flow cytometry: LEHDase, WEHDase, VEIDase, IETDase, and DEVDase. Since the relative order of caspases 3 (DEVDase) and 6 (VEIDase) in the cascade has been controversial, this caspase activation order was reexamined using confocal microscopy. The VEIDase activity appeared before DEVDase in every apoptotic cell treated with dexamethasone. In contrast, anti-Fas stimulation altered this sequence: IETDase was the first measurable caspase activity and DEVDase preceded VEIDase. In an attempt to determine the intracellular target of the potent antiapoptotic agent

carbobenzoxy-valyl-alanyl-aspartyl(beta-methyl ester)-fluoromethyl ketone (Z-VAD[OMe]-FMK), we examined its ability to inhibit previously activated intracellular caspases. However, no significant reductions of these activities were observed. These ***fluorogenic*** caspase substrates allow direct observation of the caspase cascade in intact apoptotic cells, showing that the order of downstream caspase activation is dependent on the apoptotic stimulus.

L11 ANSWER 5 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:101996 BIOSIS
DOCUMENT NUMBER: PREV200200101996
TITLE: Compositions for the detection of protease in biological samples and methods of use therefo.
AUTHOR(S): ***Komoriya, A.*** ; Parkard, B. S.
CORPORATE SOURCE: Rockville, Md. USA
ASSIGNEE: ONCOIMMUNIN, INC.
PATENT INFORMATION: US 5714342 Feb. 3, 1998
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 3, 1998) Vol. 1207, No. 1, pp. 412. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

L11 ANSWER 6 OF 7 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:62138 BIOSIS
DOCUMENT NUMBER: PREV200200062138
TITLE: Compositions for the detection of proteases in biological samples and methods of use thereof.
AUTHOR(S): ***Komoriya, A.*** ; Packard, B. S.
CORPORATE SOURCE: Rockville, Md. USA
ASSIGNEE: ONCOIMMUNIN, INC.
PATENT INFORMATION: US 5605809 Feb. 25, 1997
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Feb. 25, 1997) Vol. 1195, No. 4, pp. 2522. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

L11 ANSWER 7 OF 7 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 97:507178 SCISEARCH
THE GENUINE ARTICLE: BJ03V
TITLE: Design of profluorescent protease substrates guided by exciton theory
AUTHOR: Packard B Z (Reprint); Toptygin D D; ***Komoriya A*** ; Brand L
CORPORATE SOURCE: ONCOLMMUNIN INC, COLLEGE PK, MD 20742 (Reprint); JOHNS HOPKINS UNIV, DEPT BIOL, BALTIMORE, MD 21218; JOHNS HOPKINS UNIV, DEPT BIOPHYS, MCCOLLUM PRATT INST, BALTIMORE, MD 21218
COUNTRY OF AUTHOR: USA
SOURCE: METHODS IN ENZYMOLOGY, (MAY 1997) Vol. 278, pp. 15-23. Publisher: ACADEMIC PRESS INC, 525 B STREET, SUITE 1900, SAN DIEGO, CA 92101-4495. ISSN: 0076-6879.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 63

=> d his

(FILE 'HOME' ENTERED AT 11:21:13 ON 27 JUL 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 11:21:42 ON 27 JUL 2002
L1 5943 S FLUOROGENIC (W) (SUBSTRATE OR PEPTIDE OR COMPOSITION)
L2 456506 S PROTEASE OR PROTEINASE
L3 1780 S L1 (P) L2
L4 40842 S RHODAMINE
L5 31 S L3 (P) L4
L6 10 DUPLICATE REMOVE L5 (21 DUPLICATES REMOVED)

```
L7              2 S FLUOROGENIC (W) COMPOSITION
L8              2 DUPLICATE REMOVE L7 (0 DUPLICATES REMOVED)
L9            212 S KOMORIYA A/AU
L10            13 S L9 AND FLUOROGENIC
L11             7 DUPLICATE REMOVE L10 (6 DUPLICATES REMOVED)

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                      ENTRY     SESSION
FULL ESTIMATED COST                                   51.66       51.87

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                      ENTRY     SESSION
CA SUBSCRIBER PRICE                                   -1.86       -1.86

STN INTERNATIONAL LOGOFF AT 11:27:33 ON 27 JUL 2002
```